IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Jay BUA

Title: REDUCTION OF BREAST DENSITY WITH 4-HYDROXY TAMOXIFEN

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Applicant: Jay BUA

DECLARATION OF VALERIE MASINI-ETEVE, PH.D. UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
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Sir:

I, Valérie Masini-Etévé, do hereby declare and state as follows:

1. I am employed by Laboratoires Besins-International, the assignee of the captioned U.S. patent application, and understand that this declaration may be used to support the application.

2. My position at Laboratoires Besins-International is Head of Non Clinical R&D. I joined Laboratoires Besins-International in 1993 as a researcher working on formulations and in vitro percutaneous absorption. I received a Ph.D. in Cutaneous Biology and Pharmacology from the University of Paris XI in 1995. My responsibilities at Laboratoires Besins-International expanded in 2003 to include non-clinical, in vivo, research and development.

I. Penetration Enhancement Of 4-OHT

A. Oleic Acid

3. The Franz cell permeation studies described in this section were conducted by a contractor, Pharma Serve, Ltd., at the request of Laboratoires Besins-International. I have reviewed the report provided by Pharma Serve, Ltd., and summarize the experiments and results in the following paragraphs.

4. A preparation comprising 0.02% 4-OHT and oleic acid as a penetration enhancer was tested on rat skin samples, and the percentage of 4-OHT recovered in the receptor liquid after 48 hours was determined. Results are shown in the table below.

Ingredient (%w/w)	Formulation A	Formulation B
Oleic acid	0.00	1.00
4-OHT	0.02	0.02
Hydroxypropylmethylcellulose	2.00	2.00
Ethanol 96%	37.38	37.38
Alpha-tocopherol (an anti-oxidant)	0.10	0.10
Water	60.50	59.50
% 4-OHT recovered after 24 h	12.54 +/- 4.36	1.79 +/- 0.90

These results show that oleic acid is not an effective penetration enhancer for 4-OHT. Indeed, the preparation with oleic acid exhibited much less permeation after 48 hours than the preparation without oleic acid.

B. Isopropyl Myristate

5. The Franz cell permeation studies described in this section were conducted at Laboratoires Besins-International in 1991. I summarize the experiments and results in the following paragraphs.

6. A preparation comprising 0.02% 4-OHT and IPM as a penetration enhancer was tested on human skin samples and the percentage of 4-OHT recovered in the receptor liquid after 24 hours was determined. Results are shown in the table below.

Ingredient (%w/w)	Formulation C	Formulation D
Isopropyl myristate	0.00	1.00
4-OHT	0.02	0.02
Hydroxypropylcellulose	1.5	1.5
Ethanol	72	72
Water	Qsp	qsp
% 4-OHT recovered after 24 h	0.13 +/- 0.03	0.45 +/- 0.26

These results show that IPM is an effective penetration enhancer for 4-OHT. Indeed, the preparation with IPM exhibited 2-3 times as much permeation after 24 hours than the preparation without IPM.

II. Penetration Enhancement For Progesterone

7. The experiments described in this section were performed by me or under my direction.

8. Preparations comprising progesterone and different penetration enhancers were prepared, and permeation studies were carried out following the general protocol outlined below.

9. In vitro transdermal absorption is quantitatively studied on human ventral dermatomed biopsies placed in a static diffusion cell (Franz cell). A dermal biopsy is maintained horizontally between two parts of the cell, thus delimiting two compartments:

The upper compartment (epidermal) is made of a glass cylinder, typically having a precisely defined area of 1.77 cm^2, and is placed on the upper side of the skin

The lower compartment (dermal) is applied to the lower face of the tegument, and comprises a reservoir of fixed volume and a lateral collection port.

The two compartments are assembled via a clamp.

The lower compartment (dermal) is filled with a receptor liquid (typically sodium chloride supplemented with bovine serum). At each time point tested, the receptor liquid is entirely sampled out by the lateral collection port and replaced by fresh liquid. The lower compartment is thermostated to 37°C. Homogeneity of the temperature and the content in the receptor fluid is maintained by stirring (magnetic stirrer).

The upper compartment (epidermal) is open, exposing the epidermal surface to laboratory air.

10. For each formulation tested, multiple Franz cells are set up, with the donor skin samples distributed between the cells. Ten µl of gel are applied with a micropipette over the entire surface of the epidermis delimited by the glass cylinder of the Franz cell. Sampling from the liquid contained in the dermal compartment is carried out via the lateral collection port up to 24 hours post-application. At the end of the experiment, residual drug remaining at the surface of the skin is removed by washing the skin surface with soapy water and then rinsing. The application area is then wiped with a cotton swab. All washing media, the cotton swab and the upper part of the Franz cell are introduced into a flask with about 45 ml of ethanol, precisely weighed, and incubated overnight at room temperature in order to extract residual radioactivity. The epidermis is separated from the dermis by gentle scraping with a scalpel, and the dermis is separated from the lower part of the Franz cell. The epidermis and dermis are digested for a few hours at 60°C with 1 ml and 3 ml of Soluene 350™ (Packard), respectively, to extract radioactivity.

11. Radioactive samples are prepared for analysis as follows:

(1) The receptor liquid sampled from the lower compartment of each Franz cell is incorporated into 15 mL of liquid scintillation cocktail (Picofluor 40R, Packard).

(2) A precisely weighed aliquot of the solution containing the washing solvents is mixed with liquid scintillation cocktail Picofluor 40R.

(3) 15 ml of the liquid scintillation cocktail Hionic FLuorR is added to each sample of digested epidermis and dermis.

12. Radioactivity is measured by liquid scintillation using a Packard-tricarb 2900 TR particle counter. Results are expressed in weight or percentage of substance found in the samples with respect to the administered amount.

13. The results are set forth in the following table, and demonstrate that IPM is not the best penetration enhancer for progesterone. On the other hand, the results show that oleic acid is a more effective penetration enhancer for progesterone.

Ingredients (% w/w)	**Oleic Acid**	**Propylene Glycol**	**Isopropyl Myristate**
Progestérone	3	3	3
Oleic Acid	5	0	0
Propylene glycol	0	10	0
Isopropyl Myristate	0	0	1
Ethanol 95 %	72	72	72
Water	20	15	24
% Progesterone recovered after 24 hours	5.06 +/- 2.21	5.59 +/- 1.47	3.30 +/- 1.58

14. In another experiment with identical design, the penetration enhancer nerol and its isomer, geraniol, were tested, and proved to be valuable penetration enhancers for progesterone, leading both to a greater amount of progesterone recovered in the receptor fluid and in the dermis.

15. Also determined in these experiments was the effect on dermis retention. Oleic acid, geraniol, and nerol were found to enhance dermis retention, which may favorably influence further absorption. On the other hand, IPM had no positive effect on dermis retention.

16. Thus, of the five penetration enhancers tested, IPM appears to be among the less effective enhancers for progesterone, while oleic acid was one of the more effective ones.

17. The results reported in Sections I and II above demonstrate the unpredictability and performance variability associated with transdermal compositions in general, and with penetration enhancers in particular. As illustrated here, a penetration enhancer that is useful for one active agent is not necessarily useful for a different active agent. Oleic acid is shown above to be an effective penetration enhancer for progesterone, but is not an effective penetration enhancer for 4-OHT. Conversely, as shown above, IPM is not a particularly effective penetration enhancer for progesterone, but is an effective penetration enhancer for 4-OHT. This unpredictability means that it is not possible to modify a given composition to replace the penetration enhancer with a different penetration enhancer, and reasonably predict that the modified composition will perform equivalently to the original composition. Instead, the suitability of a particular penetration enhancer must be determined experimentally for a given composition.

18. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that willful, false statements may jeopardize the validity of the application or any patent issued thereon.

03 March 08
DATE

Valérie Masini-Etévé, Ph.D.